

Appl. No. : 10/061,438
Filed : January 31, 2002

AMENDMENTS TO THE SPECIFICATION

Please delete paragraph 1 on page 2 of the specification and replace it with a new paragraph 1 as follows:

The average red blood cell life span is 120 days, so quantitation of the percent glycation of hemoglobin has been correlated to a measure of the average glucose concentration over the previous 2 to 3 months which is a measure of glycemic control over that time period (see “Diabetes Control and Complications Trial Research Group, The effect of intensive treatment of diabetes on the development of progression of long-term complications in insulin-dependent diabetes mellitus”, New England Journal of Medicine, 329, 977-986 (1993), and “American Diabetes Association, Tests of Glycemia in Diabetes”, Diabetes Care, 20 (suppl. 1), S18-S20 ([1977]1997)).

Please delete paragraph 1 on page 6 and replace it with a new paragraph 1 as follows:

to determine total (glycated and non-glycated) bound protein. A second buffer is added to the solid support matrix which changes the conditions so that glycated protein is bound and non-glycated protein is not substantially bound and is added in an amount sufficient to rinse off unbound (non-glycated) protein. A second bound protein measurement is made. Glycated protein [in] is quantitated using the first and second bound protein measurements.

Please delete paragraph 1 on page 10 and replace it with a new paragraph 1 as follows:

The term “alkyl” refers to saturated aliphatic groups including straight-~~chair~~ chain, ~~branded~~ branched-chair and cyclic (including polycyclic) groups.

Appl. No. : **10/061,438**
Filed : **January 31, 2002**

Please delete the paragraph 2 on page 14 and replace it with a new paragraph 2 as follows:

According to an alternate aspect, the present invention is directed to methods of quantitation of glycated protein in a sample in which a solid support matrix which comprises a negatively charged group and a hydroxyboryl compound and which has a measurement area is contacted with an aliquot of a biological sample sufficient to cover the measurement area. The solid support matrix is then contacted with a first buffer which has a pH of about 5.0 to about 7.0 in an amount sufficient to rinse off unbound protein. The amount of protein bound to the measurement area is quantitated to give a first bound protein reading. The solid support matrix is then contacted with a second buffer which has a pH of about 8.0 to about 10.0 in an amount sufficient to rinse off unbound protein. The amount of protein bound to the measurement area is quantitated to give a second bound protein reading. The percentage of glycated protein in the biological sample is calculated using the first bound protein reading and the second bound protein reading.

Please delete paragraph 2 on page 15 and replace it with a new paragraph 2 as follows:

Preferably, the first and second bound protein readings involve an optical reading. More preferably, the selected property measured is absorbance or reflectance at a specified wavelength.

Please delete paragraph 1 on page 20 and replace it with a new paragraph 1 as follows:

After the sample has been absorbed into the strip, the first buffer is added. The pH of the first buffer is preferably between about 5.0 and about 7.0. More preferably, the pH of the first buffer is about 5.5 to about 7.0 for quantitation of glycated hemoglobin and about 5.0 to about 6.5 for quantitation of glycated ~~hemoglobin~~ albumin. The buffer contains a suitable buffering agent which has a pKa appropriate for control of the pH within the given range, but that does not otherwise affect the binding of the protein. Buffering agents suitable for use in the first buffer are known to those of skill in the art. Preferred buffering agents for use in the first buffer include

MES, MOPS and HEPES. The buffering agent is present in a concentration sufficient to maintain the pH in the desired range. Suitable concentrations for the buffering agent may range from about 5mM to about 500mM. If the biological sample includes red blood cells, the buffer may further comprise a cell lysing agent such as, for example, Triton X-100 or Igepal CA-630. The buffer volume added can be standardized to a specific number of drops adequate to rinse through excess, non-bound sample. The adequacy of rinsing can also be monitored by the reflectance meter taking multiple measurements with the final measurement taken when the change is essentially zero.

Please delete the second full paragraph on page 24 of the specification, the paragraph beginning "The binding . . .," and replace it with a new paragraph as follows:

The binding characteristics of the boronated solid support matrix prepared as above using different soak times [was] were measured using the elution assay described in Example 2. The resulting binding of total (open triangles) and glycated hemoglobin (closed triangles) is depicted in Figure 1. Open circles show the ratio of glycated to total hemoglobin.

Please delete the second paragraph on page 25 and replace it with the following amended paragraph:

The following assays were performed to compare the two methods. In the Single Measurement Method assay, blood lysate samples from a non-diabetic individual and a diabetic individual were diluted 1:1 with 500mM ammonium acetate buffer pH=9.5 with 50mM Mg^{++} and the samples were allowed to be in contact with the boronated solid support matrix prepared as in Example 1 for a variety of time periods. Following incubation, the boronated solid supports were rinsed with ammonium acetate buffer pH=9.5. Glycated hemoglobin was eluted with an elution buffer comprised of tris buffer at pH 8.0 containing 200mM sorbitol. The absorbance of the eluent was measured at 415mM.